

ESTIMATION OF HISTAMINE AND LEUKOTRIENE D4 AS INFLAMMATORY MARKERS IN PATIENTS WITH ASTHMA

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ABSTRACT

This study aimed to estimate the concentrations of histamine and leukotriene D4 by ELISA in patients with asthma, and to indicate the relationship between the disease and the gender in Babylon province, Iraq. Results revealed that there was a significant difference ($p<0.05$) in concentration of histamine(ng/ml) between males and females of patients in 21-40 years group. No significant differences were found between both sexes in 1-20 and 41-60 years groups. Meantime, the results illustrated that both males and females showed no significant differences($p<0.05$) in concentration of leukotriene D4(ng/ml) in all age categories. Furthermore, the study revealed that the concentration of leukotriene D4 showed significantly increase ($p<0.05$) in the first and second age groups compared with the third age group of patients and healthy individuals. The results displayed that there was decrease ($p<0.05$) in the level of histamine in first and third age categories of patients in comparison with control group, also there was no significant differences in levels of this inflammatory mediator between groups of patients.

KEYWORDS: Patients, Asthma, Leukotriene D4, Histamine, ELISA

INTRODUCTION

Asthma is a life threatening chronic disease of the respiratory system. It has been very common among children and adults (Masoli et al., 2009). It about to 200 - 300 million patients around the globe are suffering from that disease and the number is rising every year (Manohar and Selvakumaran, 2012). Amin et al (2005) reported that there is an increase in mast cells degranulation in atopic asthmatics resulting from an IgE – dependent activation of the cells. Furthermore, a variety of mast cell products can be released upon degranulation (Robinson, 2004), such as histamine and leukotrienes may play a role in airway smooth muscle cells proliferations and remodeling (Holgate et al., 2003). Leukotrienes (LTs) are lipid mediators involved in airway inflammation and certain clinical features of asthma (Hallstrand and Henderson, 2010). Leukotrienes comprise a family of products of the 5-lipoxygenase pathway of arachidonic acid metabolism (Marco and William, 2007). There are two families of leukotrienes, the first group LTsB4 (dihydroxy leukotriene) acts primarily in conditions in which inflammation is dependent on neutrophils, while the second group cysteinyl leukotrienes including CysLTC4, D4, E4 and F4 is concerned primarily with eosinophils and mast cells induced bronchoconstriction in asthma (Duroudier et al., 2009). LTD4, is found on leukocytes, smooth muscle cells and endothelial cells, and many have a role in fibrosis and vascular injury (Beller et al., 2004; Uzonyi et al., 2006). The aim of this study was to examine the influence of histamine and CysLTsD4 as inflammatory markers in patients with bronchial asthma in Babylon governorate, Iraq.

MATERIALS AND METHODS

Patients and Control

The work was applied on 60 asthma patients (27 males and 33 females) admitted to the center of asthma and allergy, maternity and pediatrics hospital as well as Marjan teaching hospital in Babylon province, and 30 apparently subjects(14 males and 16 females) with no symptoms of asthma were selected as control group. The cases of this study were divided into three age categories for comparison: (1-20) years, (21-40) years and (41-60) years. The numbers of cases were 15, 18 and 27 in each group respectively. The diagnosis for patients was based on the pre- diagnosis by physicians.

Blood Samples

The blood samples were drawn from each patient (admitted to the hospitals) and control (5 ml) by vein puncture using disposable syringes. The blood was placed in disposable tube, kept to clot at room temperature, and then centrifuged at 3000 r.p.m (Bishop et al., 1985) for 10 minutes, after that sera samples were carefully transferred to eppendorf tubes and stored in aliquots at deep freezing until used.

Immunological Tests

The concentrations of histamine and leukotriene D4 were estimated by ELISA according to the manual procedure of Creative – Diagnostic Company(USA).

Statistical Analysis

The results were analyzed using statistical system SPSS version -18 (T-testing).

Results

The results showed that there was a significant differences ($p<0.05$)in the level of histamine between males and females in the second age category of asthmatic patients, while there is no significant differences between both gender in the first and third age categories of patients as revealed in figure 1, while the results showed no significant differences ($p<0.05$) in concentration of leukotriene D4 between males and females in all age categories of patients (Figure 2).

The results illustrated that there was increase ($p<0.05$) in mean of concentration of leukotriene D4 in the first and second age categories of patients (which reached 36.39 and 49.63 ng/ml respectively) compared with the third group of patients and control groups, also there is difference in level of leukotriene D4 between patients in the first and second age groups.

The concentration of histamine showed a significant decrease ($p<0.05$) in the first and third age categories of patients in comparison with healthy subjects which reached 15.74, 11.1, 25.53 and 25.6 ng/ml respectively, while there were significant differences between age groups of patients as showed in (Table 1).

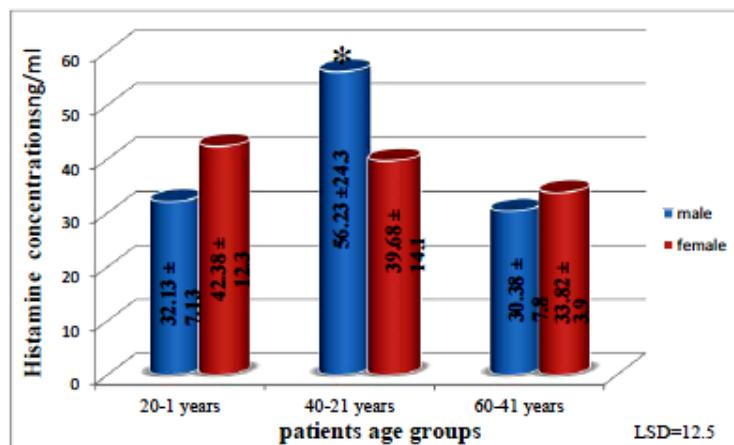


Figure 1: The Relationship between Sex and Concentration of Histamine among Asthmatic Patients of different Age Groups

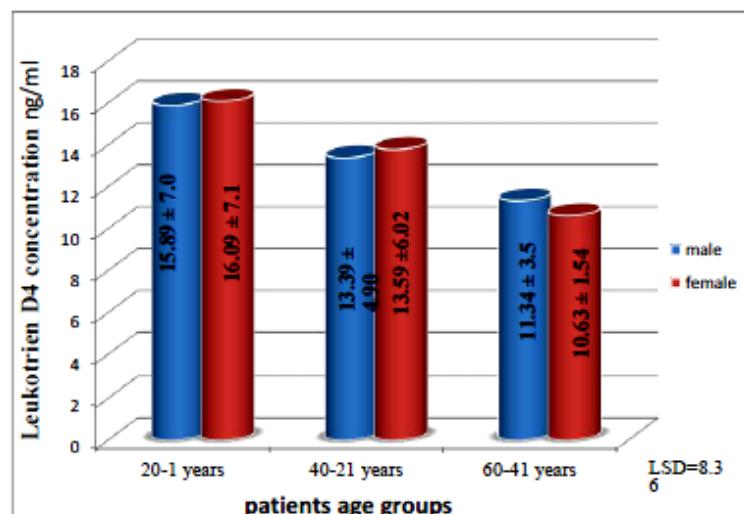


Figure 2: The Relationship between Sex and Concentration of Leukotriene D4 among Asthmatics Patients of different Age Groups

Table 1: The Concentrations of Histamine and Leukotriene D4 in Patients with Asthma at Different Age Categories

Parameters		Leukotriene D4(ng/ml) Mean ± S.D	Histamine(ng/ml) Mean ± S.D
Age Categories			
1-20 years	Patients	36.39 ± 9.44*	15.74 ± 2.24*
	Control	28.6 ± 3.56	25.53 ± 12.31
21-40 years	Patients	49.63 ± 19.22*	14.34 ± 2.13
	Control	25.26 ± 4.9	10.21 ± 0.63
41-60 years	Patients	27.71 ± 7.76	11.10 ± 1.84*
	Control	27.30 ± 5.41	25.60 ± 4.57
LSD	6.7		7.3

(*): significant differences

DISCUSSIONS

The results showed that there is no significant differences in the level of histamine between both sex in the first and third age categories, while there was a significant decrease in mean of concentration of histamine in the first and third

age groups of patients. These results were inconsistent with the study of EL-Zohery et al., (2012) who reported that the level of histamine, total serum IgE, and specific immunoglobulin E (SIgE) were significantly higher in the asthmatics patients than in the healthy individuals. Furthermore, it has been proven that individuals with an allergic disease, the blood level of chemical mediators, such as histamine is increased (Choi et al., 1988; Kenneth et al., 2012). But, Tuula (2005) illustrated that histamine concentration in the asthma group did not differ from healthy control, while in the periodontitis group a significant decrease in gingival histamine concentrations was found, also treatment with an antihistamine lowers plasma histamine (Fremin et al., 1986). Furthermore, Eric and Ed Weeissberg (1987) found that patients with elevated IgE frequently have extremely low blood histamine. Sehgal et al. (2013) illustrated that histamine when inhaled causes hypoxia and leads to convolution in the guinea pigs and causes very strong smooth muscle contraction and capillary dilation in the cardiovascular system.

The study showed no significant differences ($p < 0.05$) in the level of cysteinyl leukotriene D4 between both sexes in all age groups of patients, whereas the concentration of this allergic mediator revealed a significant increase in the first and second age groups of patients. These data are consistent with the results of many of the researchers pointed out that increasing the level of this mediator in asthmatic patients. David and Michael (2008) revealed that CysLTs roles specifically LTD4 is to trigger contractions in the smooth muscle lining the bronchioles; their overproduction is a major cause of inflammation in asthma and allergic rhinitis. Furthermore, the levels of CysLTs have been reported to be increased in patients with asthma, correlating with disease severity (Samitos et al., 2009). CysLTS C4 and D4 are 1000- fold more potent than histamine (Christie et al., 1993); in addition, increased CysLTs concentrations have been detected in the sputum of patients with this and have been shown to correlate with symptom severity (Pavord et al., 1999), therefore, there is an important role for cysteinyl leukotrienes in the pathogenesis of airway inflammation and bronchoconstriction in asthma (Mehrota and Henderson, 2009; EL-Akkary et al., 2013).

CONCLUSIONS

There were significant decreases in the level of histamine in the first and third age groups of asthmatic patients compared with control group. The concentration of leukotriene D4 showed a significant increase in the first and second age groups of patients compared with healthy subjects. The differences between results in this study and other studies may be related to the variation in genetic and environmental factors between popular communities.

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